Morphology of adult rat urinary bladder after ovariectomy and the role of Tibolone administration

Abeer M. Azmy and Maha A. Abdallah

Histology and Cell Biology Department, Faculty of Medicine, Zagazig University. Egypt.
maha_amine70@yahoo.com

Abstract: Introduction: Estrogen has been implicated as an important endogenous compound for maintaining lower urinary tract function. **Aim of the study:** This work aimed to detect the effect of estrogen hormone deprivation by bilateral surgical ovariectomy on urinary bladder morphology and the role of Tibiolone administration. **Material and methods:** Twenty four adult albino rats were equally divided into three groups; control (I), ovariectomized (II) and Tibolone treated ovariectomized (III) groups. Rats in groups II and III were left for 3 weeks after ovarictomy. After this duration, group III rats were treated orally with 0.25mg Tibolone /kg/day for continuous 12 weeks. Animals' bladder were dissected out and processed for examination by light and electron microscope. The area percentages of collagen fiber, smooth muscle with their ratio as well as bladder wall and urothelium thickness were estimated and statistically analyzed. **Results:** Urinary bladder of the ovariectomized rats revealed an observable focal reduction in the urothelium and total bladder wall thickness. Many epithelial cells showed distortion with indistinct cell junctions, wide intercellular spaces and cellular infiltration. Musculosa had wide separation of their bundles with abundant collagen fibers inbetween. Numerous myocytes had indistinct dense bodies, plaques and corrugated sarcolemmae. Most of these changes were improved with Tibolone treatment. Estimated and analyzed urothelium and bladder wall thickness as well as area percentages of collagen fiber, smooth muscle with their ratio confirmed the results. **Conclusion:** Estrogen deficiency led to deterioration of bladder morphology. Tibolone is considered a good therapy for estrogen deficiency in improving bladder morphology.

[Abdullah Abeer M. Azmy and Maha A. Abdallah. **Morphology of adult rat urinary bladder after ovariectomy and the role of Tibolone administration**. *J Am Sci* 2013;9(7):419-431]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 52

Key words: ovariectomy – urinary bladder-estrogen deficiency – Tibolone- ultrastructure.

1. Introduction

Ovary is the primary female reproductive organ that is responsible for not only ova production but also estrogen and progesterone hormones formation. Although estrogen and progesterone receptors are present in the female urogenital tract, serum level of estrogen had been implicated in the urogenital tract function**[1,2]**. Estrogen affects growth, differentiation, and function of mammary glands, uterus, vagina and ovaries. It also plays important roles in maintaining bone density and it is thought to be cardioprotective, largely through its effects on blood lipids **[3]**. Physiological alterations in circulating estrogen level can be detected during menstruation, pregnancy and menopause **[4].**

Menopause is a physiological cessation of the monthly cycle in women without an obvious intervening cause as exogenous hormone use, dietary deficiencies or surgical removal of the uterus or ovaries. It is a consequence of permanent cessation of the primary functions of the ovaries. The average age of natural menopause has consistently been estimated between 50 and 51 years**[5,6]**.

However, the human ovaries may stop working at early age before the age 40 that known as premature ovarian failure (POF) or premature menopause. This condition is biochemically characterized by low levels of gonadal hormones and high levels of gonadotropins. Premature menopause is usually idiopathic in about two third of cases. In the remaining cases, it may be surgically induced as in unilateral or bilateral ovariectomy or secondary to autoimmune disorders, chemotherapy, radiotherapy or diabetes mellitus**[7-9]**.

Regardless the cause, an early decline in estrogen production can be translated into a substantially greater risk. It may cause low bone density, fractures, impaired endothelial function, coronary heart disease, Alzheimer's disease and lower urinary tract symptoms. Approximately 80% of women experience menopausal symptoms and among them about 20% have severe symptoms **[5,10-13].**

A high incidence of urogenital symptoms as urgency, frequency, nocturia, incontinence and urinary tract infection during menopause are seen. About two third of women don't relate their urinary complaints to the menopause **[1,6,14,15]**.

Tibolone is a synthetic steroid used as hormonal replacement therapy (HRT) to reduce the distressing symptoms in menopause. After ingestion, it is converted to three metabolites; 3 alpha and 3 beta hydroxyl Tibolone which have estrogenic effects in addition to delta 4 isomerase, which has progestogenic and androgenic properties **[16,17]**. It may have

favorable effects on bone, vagina, climacteric symptoms and mood in postmenopausal women without having an estrogen-like stimulating effect on the endometrium or breast **[18]**.

Few searches tried to correlate the clinical symptoms of bladder with low sex steroid hormones. So, this search was done to detect the effect of estrogen hormone deprivation by bilateral surgical ovariectomy on urinary bladder morphology and the role of Tibiolone administration.

2. Material and Methods

Twenty four healthy adult albino rats weighing 180-200 g were used in this study. They were housed in stainless-steel cages and were maintained in room temperature. They were allowed water *ad-libitum* and were fed a standard diet. They were equally divided into three groups (8 animals each); control (I) group, ovariectomized (II) group and Tibolone treated ovariectomized (III) one. The control group is further subdivided into two equal subgroups; rats without any surgical procedure; non-operated (Ia) and s ham operated rats (Ib).

The operated rats of groups II and III were anesthetized with 50 mg per kilogram body weight sodium pentobarbital intraperitoneally. Their abdomens were shaved and sterilized with betadine. Under sterile condition, a small vertical midline incision was made in the lower abdomen to explore the lower abdominal cavity. Both ovaries were totally removed and the incision was closed by silk suture. In sham operated subgroup (Ib), the same procedure was performed without ovariectomy **[19]**. After 3 weeks of the operation, rats of group III were treated orally with 0.25mg Tibolone /kg/day (ADWIA Company) for continuous 12 weeks **[20]**.

At the end of the experiment, the rats of all groups were anesthetized with 50 mg sodium pentobarbital per kilogram body weight intraperitoneally and then intracardiac perfusion was done by 2.5% glutaraldhyde buffered with 0.1 M phosphate buffer at pH 7.4 for partial fixation of the urinary bladder. The bladder of each animal was dissected out carefully and punctured with a needle to remove the urine. Then it was processed for light and electron microscopic examinations. Specimens for light microscope examination were fixed in 10% neutral formol saline for 24 hours and were processed to prepare 5 µm thick paraffin sections for Haematoxylin & Eosin and Mallory's Trichrome stains **[21]**.

Specimens for electron microscope examination were immediately fixed in the same perfusion fixative (2.5 % glutaraldehyde) for 2 hrs and postfixed in 1% osmium tetroxide buffered with 0.1 M phosphate buffer at pH 7.4 for 1 h. Then, they were dehydrated in ascending grades of alcohol and embedded in resin to prepare semithin sections and ultrathin sections using a Leica ultracut (UCT) (Glienicker, Berlin, Germany). Semithin sections (1 µm thick) were stained with 1% toluidine blue for light microscope examination **[21]**. Ultrathin sections were stained with uranyl acetate and lead citrate **[22]**.They were examined with a JEOL JEM 1010 transmission electron microscope (Japan) in the Electron Microscope Research Laboratory (EMRL) of the Histology and Cell Biology Department, Faculty of Medicine, Zagazig University (Egypt).

Morphometric study:

The image analyzer computer system Leica Qwin 500, UK in the Histology Department, Faculty of Medicine, Cairo University, was used to evaluate the bladder wall and urothelium thickness in millimeter and micrometer respectively using the interactive measure menu. The procedure was performed using H&E stained sections. Also, the mean area percentages of collagen fiber (CF) and smooth muscle (SM) within the musculosa were detected using the Mallory's trichrome-stained slides. Ten non overlapping high-power fields (X400) from each slide of five animals of each group were used.CF/SM ratio was calculated.

Statistical analysis:

Data for all groups were expressed as mean ± standard deviation (X±SD). The data obtained from the image analyzer were subjected to SPSS programme version 15. Statistical significant difference was determined by one way analysis of variance (ANOVA). The probability values (P) < 0.05, < 0.001 and > 0.05 were considered significant, highly significant and non-significant respectively.

3.Results

Morphological results:

Light microscope examination of the sections in empty urinary bladder of the control adult rats (Ia and Ib) showed that its' wall was formed of mucosa, musculosa and serosa. The mucosa was thrown into numerous thick folds **(Fig. 1)**.It was composed of multilayered transitional epithelium; urothelium and subepithelial connective tissue; lamina propria. The latter was arranged into two zones, superficial dense zone containing many cells and deep lighter zone with less cellular element **(Fig. 2)**. The urothelium was arranged into basal cuboidal, intermediate polyhedral and superficial dome shaped cells **(Fig.3)**. Musculosa was the thickest layer. It was formed of smooth muscle bundles arranged in various directions. The inner and outer layers of myofibers were arranged longitudinally while the middle one was circularly arranged. The muscle fibers appeared as spindle shaped cells with

central oval nuclei. They were tightly adhering to each other **(Figs. 1&4)** with minimal collagen fibers in between muscle bundles **(Fig. 5)**.

Electron microscope examination of the ultrathin sections of the same group showed that all urothelial cells had irregular euchromatic nuclei. The basal cells were small while intermediate ones were larger and elongated than the basal. Superficial cells were overlying on two or three deeper cells **(Fig. 6)**. Their adjacent lateral surfaces were joined by tight junction with prominent interdigitations. Luminal surfaces were angular in outline. Their cytoplasm contained variable sized fusiform vesicles and lysosomes **(Fig. 7)**. Smooth muscle cells (myocytes) had many dense bodies in the sarcoplasm. Their sarcolemmae appeared straight with numerous plaques. Among muscle cells, few collagen fibers were present within the minimal intercellular spaces **(Fig. 8)**.

Light microscope examination of the sections in empty urinary bladder of the ovariectomized adult rats revealed an observable focal reduction in the epithelial lining and the total thickness of the bladder wall in comparison with that observed in the control group in Figs 1&2 **(Fig. 9)**. Some epithelial cells had vacuolated cytoplasm with peripherally placed nuclei. Few cells had deeply stained nuclei. Desquamated epithelial cells were observed within the lumen. Areas of complete denudation of the mucosa with exposed lamina propria were also encountered **(Fig. 10)**. Musculosa was distorted in arrangement. It showed wide separation of the muscle bundles. Most of muscle cells were darkly stained with markedly corrugated cell membrane**(Fig. 11)**.Abundant collagen fibers were observed among muscle bundles **(Fig. 12)**.

Electron microscope examination of the sections in empty urinary bladder of the same group showed marked reduction in the urothelium layers **(Fig. 13)** with indistinct cell junctions**(Fig. 14)** and wide intercellular spaces**.** Infiltrating inflammatory cells contained numerous variable sized electron dense granules were detected in urothelium**(Fig. 15)**.Most of the myocytes showed indistinct dense bodies and plaques**.** Their sarcolemmae were markedly corrugated with widened intercellular spaces containing abundant collagen fibers**(Fig. 16)**.

Light microscope examination of the sections in empty urinary bladder of the Tibolone treated ovariectomized adult rats showed that urothelium as well as total bladder wall thickness was relatively increased when compared with ovariectomized group **(Fig. 17).** Although the urothelium became multilayered, its cells were disorganized containing variable sized vacuoles **(Fig. 18)**. The smooth muscle bundles appeared in different directions. Most of smooth muscle cells had pale stained cytoplasm. Few ones were still deeply stained with corrugated outlines**(Fig. 19)**. Some collagen fibers were observed among muscle bundles **(Fig. 20)**.

Electron microscope examination of the sections in the empty urinary bladder of the same group showed that all urothelial cells were nearly close to each other containing many vacuoles **(Fig. 21)**. The adjacent lateral surfaces of superficial cells were joined by tight junction with prominent inter digitations **(Fig. 22)**. Myocytes had straight sarcolemmae with distinct dense bodies and plaques**.** Sarcolemmae of some myocytes were corrugated with minimal intercellular spaces containing some collagen fibers**(Fig. 23)**.

Morphometrical and statistical results

Statistical analysis of the urothelium and bladder wall thickness showed decrease in ovariectomized group as compared with the control one. However, both thickness were increased in Tibolone treated ovariectomized group as compared with the ovariectomized one approaching control level **(Tables 1,2)**

Morphometrical and statistical analysis revealed decrease in area % of smooth muscle (SM) with increase in area % of collagen fiber (CF) and CF/SM ratio in ovariectomized group as compared with control one. However, the area % of SM was increased with decrease in area % of CF and CF/SM ratio in Tibolone treated ovariectomized group as compared with the ovariectomized one approaching control level**(Table 3)**.

Table (1): Comparison between urothelium thickness in the different studied group using ANOVA test.

	X±SD	F	*P*. value
Control	**44.72±11.23**	**5.86**	***0.0069**
Ovariectomy	**30.12±7.48**		
Tibolone treated	**39.27±9.82**		

Table (2): Comparison between bladder wall thickness in the different studied group using ANOVA test.

	X±SD	F	*P*. value
Control	**1.17 ± 0.10**	**9.54**	****0.000591**
Ovariectomy	**0.95 ± 0.13**		
Tibolone treated	**1.09 ± 0.11**		

Table (3): Comparisons between the mean area percentages of collagen fiber, smooth muscle and CF/SM ratio using ANOVA test.

	Control	Ovariectomy	Tibolone treated	F	P. value
	Mean ±SD	Mean ±SD	Mean ±SD		
CF	**0.1604±0.0401**	**0.2086±0.0415**	**0.1763±0.0265**	**4.52**	***0.018**
SM	**0.5146±0.0670**	**0.4035±0.0467**	**0.4907±0.0473**	**11.79**	****0.00015**
CF/SM	**0.3189±0.0865**	**0.5378±0.0848**	**0.3746±0.0630**	**21.1**	****0.000002**

* Significant (p <0.05) as compared with control group.
** Highly significant (p <0.001) as compared with control group.

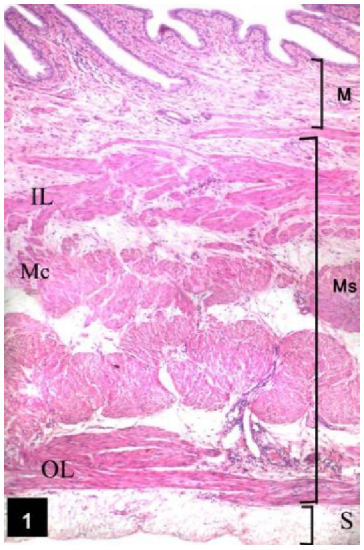

Figure (1): A photomicrograph of a section in the urinary bladder of the control adult rats showing mucosa (M), musculosa (Ms) and serosa (S) of the bladder wall. The mucosa is thrown into numerous thick folds. Musculosais the thickest layer arranged in inner longitudinal (IL), middle circular (Mc) and outer longitudinal (OL) layers. (H&E X 100).

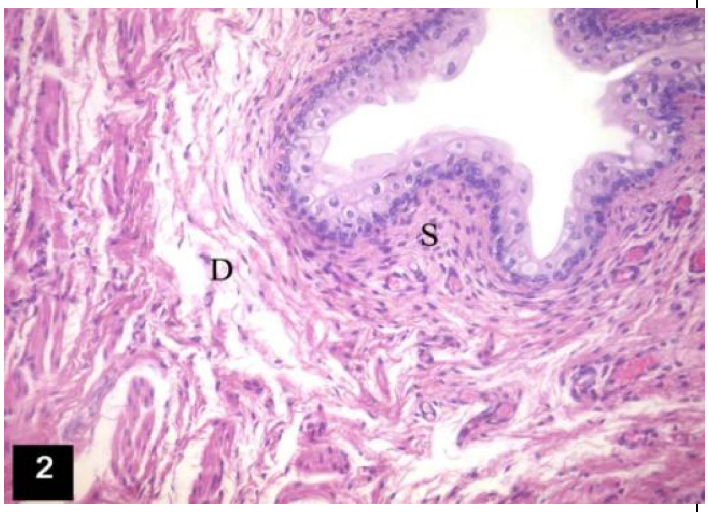

Figure (2):A photomicrograph of a section in the urinary bladder of the control adult rats showing urothelium and subepithelial connective tissue with its two zones. Superficial (S) dense zone contain many cells and deep (D) lighter zone has less cellular element. (H &E X200).

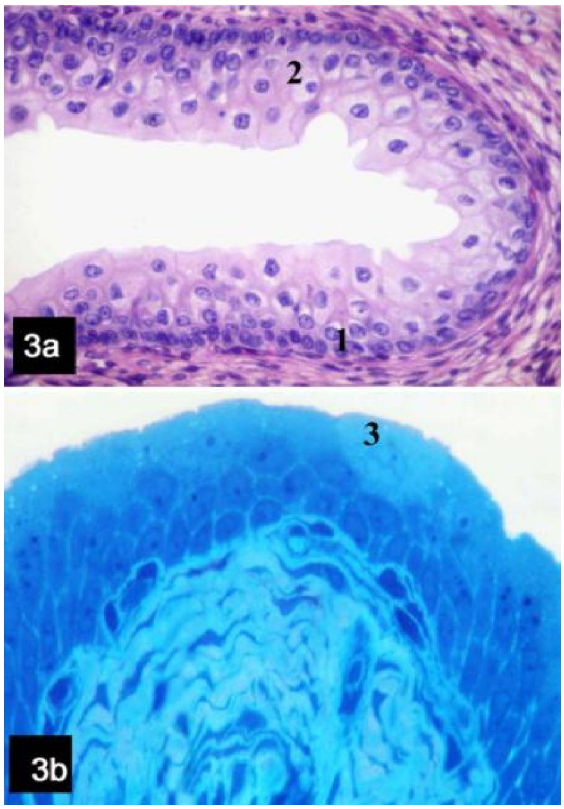

Figure (3): A photomicrograph of a section in the urinary bladder of the control adult rats showing the basal cuboidal (1), intermediate polyhedral (2)(a: H&E X 400) and superficial dome (3) shaped cells of the urothelium (b:Toluidine blue X 1000).

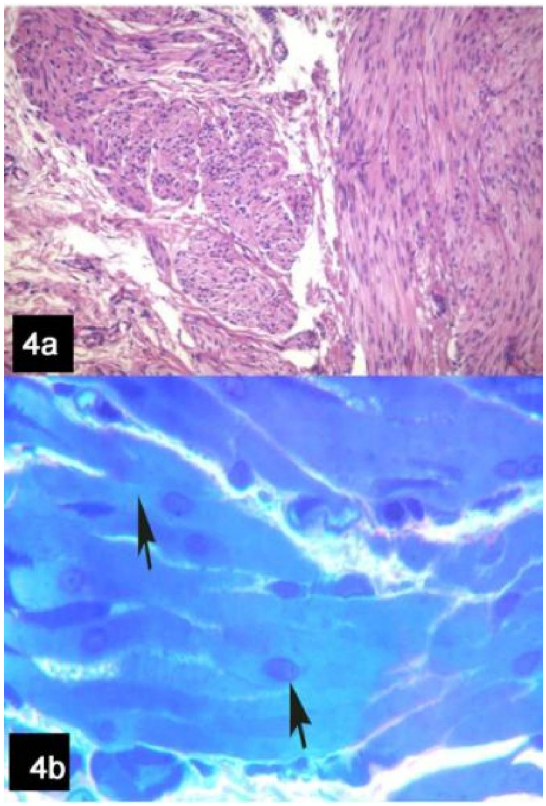

Figure (4): A photomicrograph of a section in the urinary bladder of the control adult rats showing various bundles directions of the smooth muscle fibers (a: H&E X 100). These fibers(arrows) appear spindle shaped with central oval nuclei and tightly adhering to each other (b: Toluidine blue X1000).

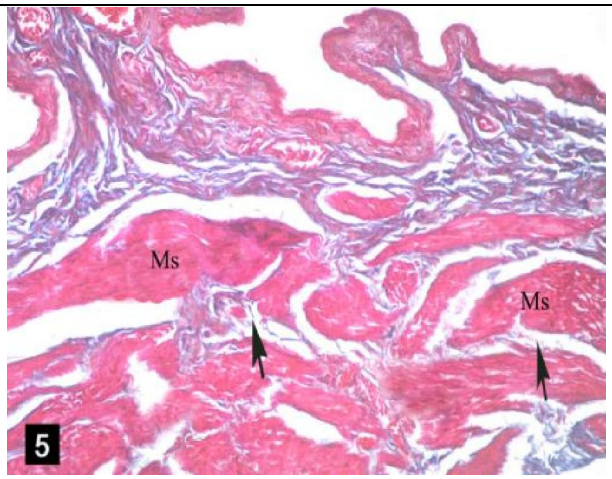

Figure (5): A photomicrograph of a section in the urinary bladder of the control adult rats showing minimal collagen fibers (arrows) in between muscle bundles (Ms). (Mallory's trichrome: X200).

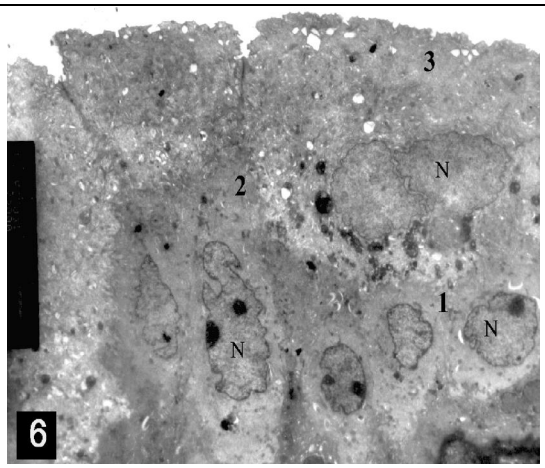

Figure (6): An electron micrograph from the control adult urinary bladder showing irregular euchromatic nuclei (N) of urothelial cells. The basal (1),intermediate (2)superficial urothelial cells (3) are noticed (X4000).

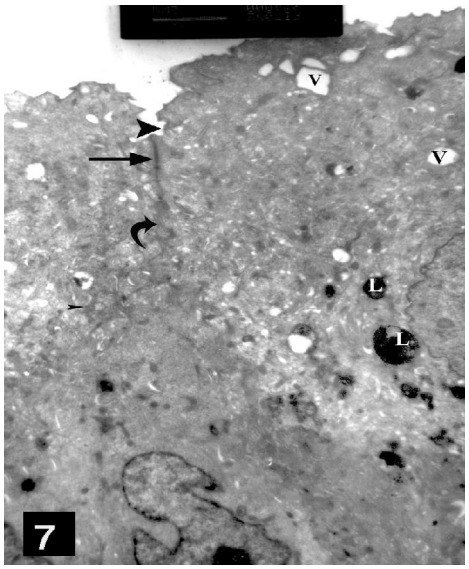

Figure (7): An electron micrograph from the control urinary bladder showing tight junction (arrow) with prominent interdigitations (curved arrow) of the adjacent lateral surfaces of superficial cells. The luminal surfaces are angular (arrow head) in outline. Their cytoplasm contains variable sized fusiform vesicles (V) and lysosomes (L). (X 9000).

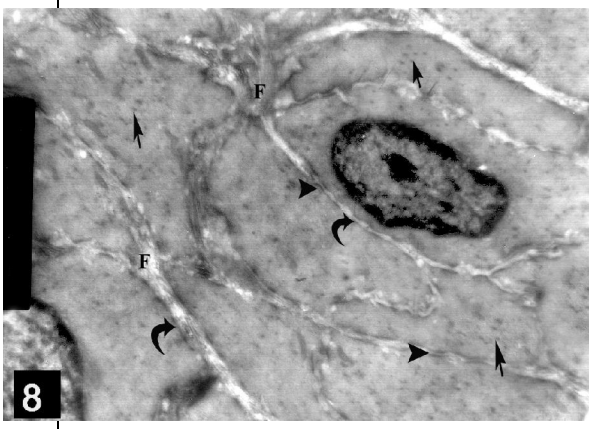

Figure (8): An electron micrograph from the control urinary bladder showing many dense bodies (arrows) in the sarcoplasm of myocytes. The sarcolemmae appear straight (curved arrow) with numerous plaques (arrow heads). Among myocytes, few collagen fibers (F) are present within minimal intercellular spaces. (X11000).

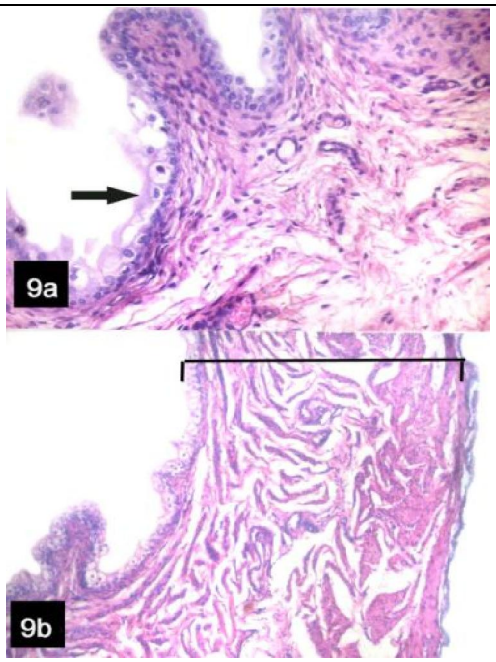

Figure (9): A photomicrograph of a section in the urinary bladder of the ovariectomized adult rats showing an observable focal reduction in the epithelial lining (arrow)(a: H&E X200) and the total thickness of the bladder wall (b: H&E X100) in comparison with that observed in figs. 1&2.

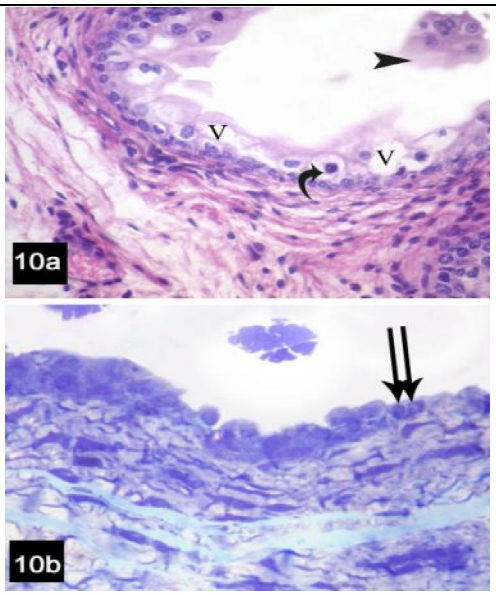

Figure (10): A photomicrograph of a section in the urinary bladder of the ovariectomized adult rats showing vacuolated (v) cytoplasm with peripherally placed nuclei in some epithelial cells. Few cells have deeply stained nuclei (curved arrow). Desquamated epithelial cells (arrow head) are observed within the lumen (a: H&E X400). Area of complete denudation (double arrows) of the mucosa with exposed lamina propria is also encountered (b: Toluidine blue X1000).

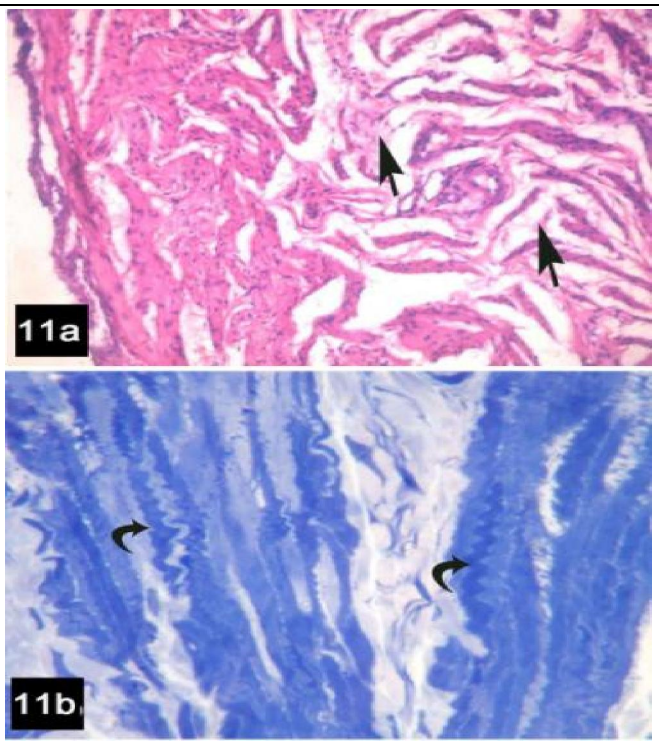

Figure (11): A photomicrograph of a section in the urinary bladder of the ovariectomized adult rats showing distorted arrangement of the musculosa with wide separation of its muscle bundles (arrows) (a: H&E X400). Most of muscle cells are darkly stained with markedly corrugated cell membrane (curved arrow) (b: Toluidine blue X1000).

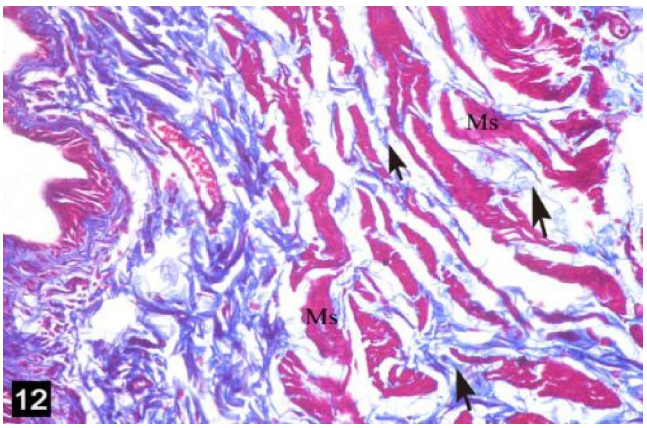

Figure (12): A photomicrograph of a section in the urinary bladder of the ovariectomized rats showing abundant collagen fibers (arrows) in between muscle bundles (Ms). (Mallory's trichrome: X200).

Figure (13): An electron micrograph from the urinary bladder of the ovariectomized rats showing the marked reduction in the urothelium layers(arrow). (X 8500).

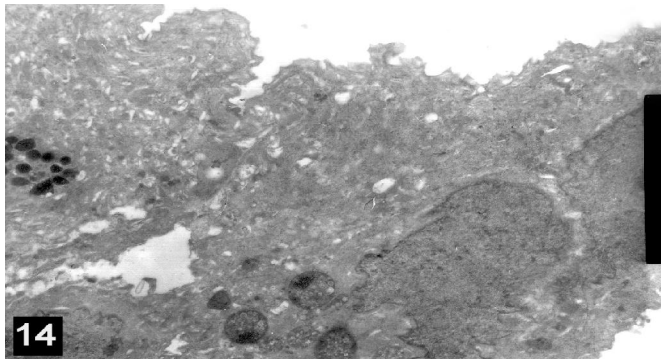

Figure (14): An electron micrograph from the urinary bladder of the ovariectomized rats showing indistinct cell junctions of the urothelial cells.(X9000).

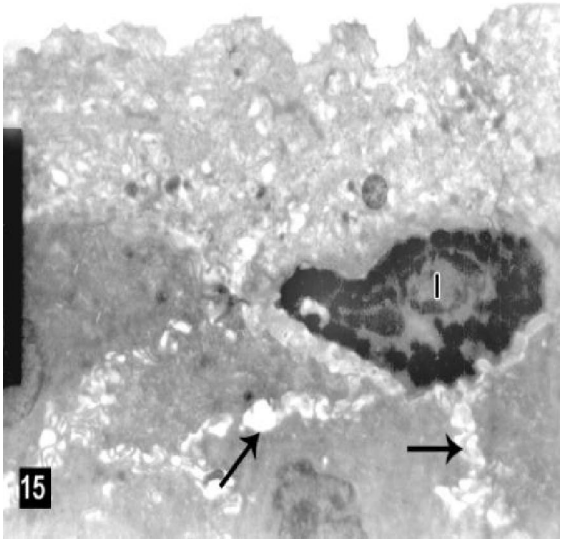

Figure (15): An electron micrograph from the urinary bladder of the ovariectomized rats showing wide intercellular spaces of the urothelium (arrows) with infiltrating inflammatory cell (I) contained numerous variable sized electron dense granules. (X8500).

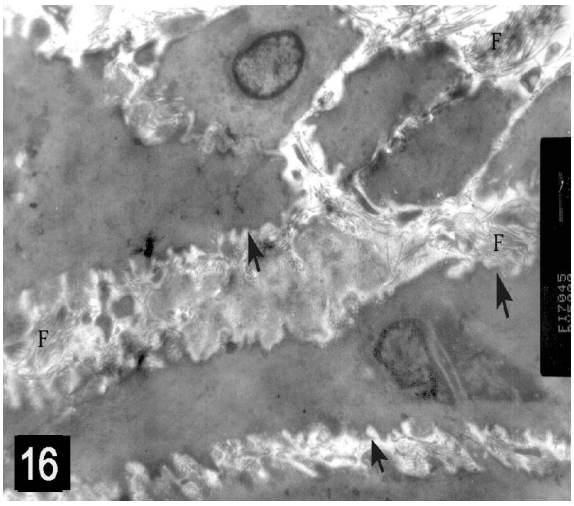

Figure (16): An electron micrograph from the urinary bladder of the ovariectomized rats showing indistinct dense bodies and plaques in myocytes**.** Their sarcolemmae are markedly corrugated (arrows) with widened intercellular spaces containing abundant collagen fibers (F). (X11000).

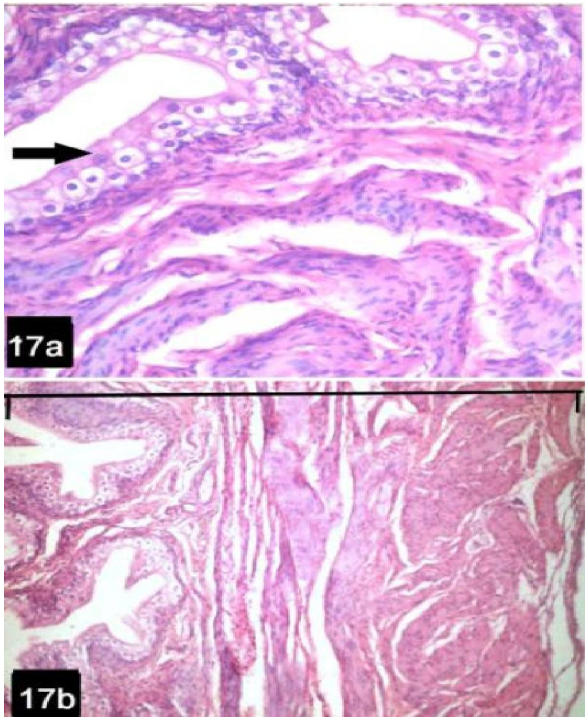

Figure (17): A photomicrograph of a section in the urinary bladder of the Tibolone treated ovariectomized adult rats showing that urothelium (arrow) (a: H&E X200) as well as total bladder wall thicknessare relatively increased (b: H&E X100) when compared with Fig. 9.

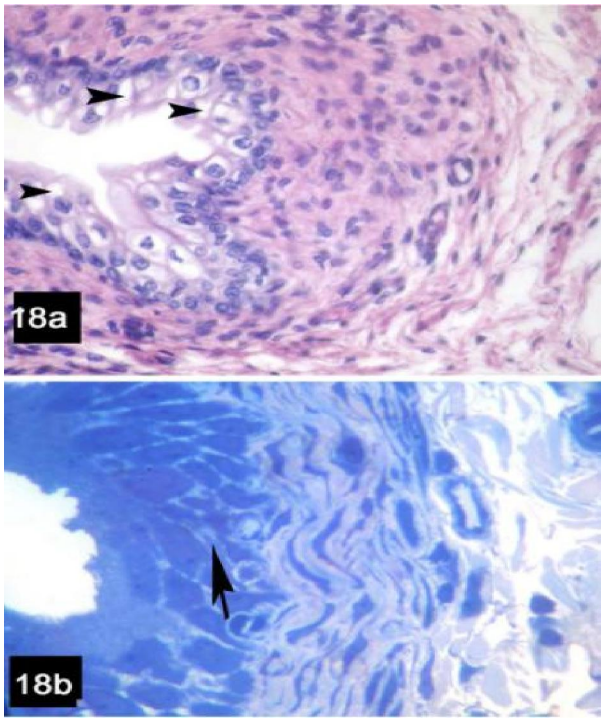

Figure (18): A photomicrograph of a section in the urinary bladder of the Tibolone treated ovariectomized adult rats showing the multilayered of urothelium. Its cells are disorganized (arrow) containing variable sized vacuoles (arrow heads). (a: H&E X 400 & Toluidine blue X1000).

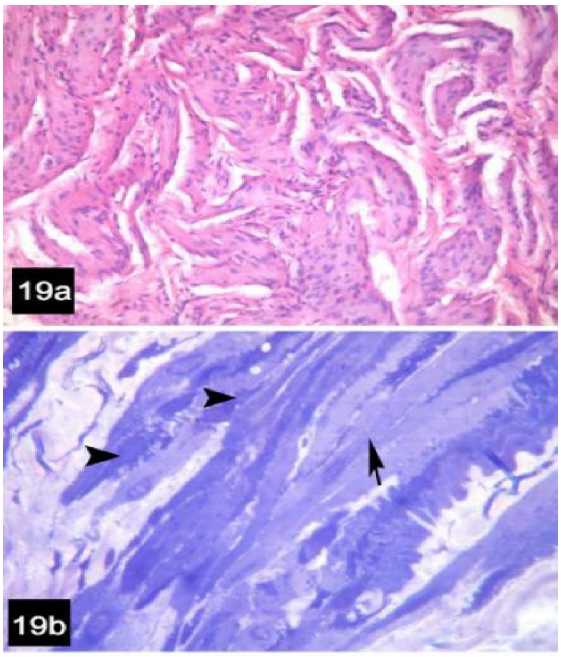

Figure (19): A photomicrograph of a section in the urinary bladder of the Tibolone treated ovariectomized adult rats showing that the smooth muscle bundles appear in different directions. (a: H&E X 400). Most of smooth muscle cells have pale stained cytoplasm (arrow). Few ones are deeply stained with corrugated outline (arrow heads) (b: Toluidine blue X 1000).

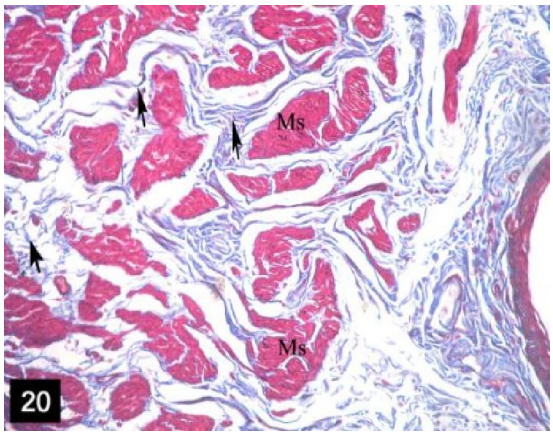

Figure (20): A photomicrograph of a section in the urinary bladder of the Tibolone treated ovariectomized rats showing some collagen fibers (arrows) in between muscle bundles (Ms). (Mallory's trichrome: X200).

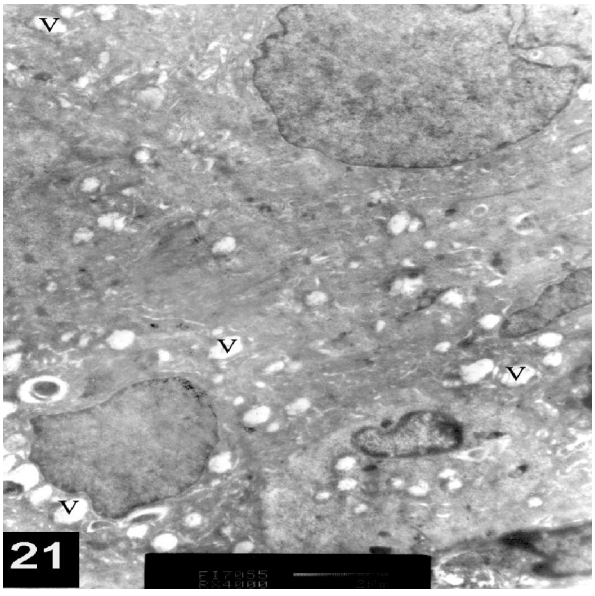

Figure (21): An electron micrograph from the urinary bladder of the Tibolone treated ovariectomized rats showing that all urothelial cells are nearly close to each other containing many vacuoles (v) (X 9000).

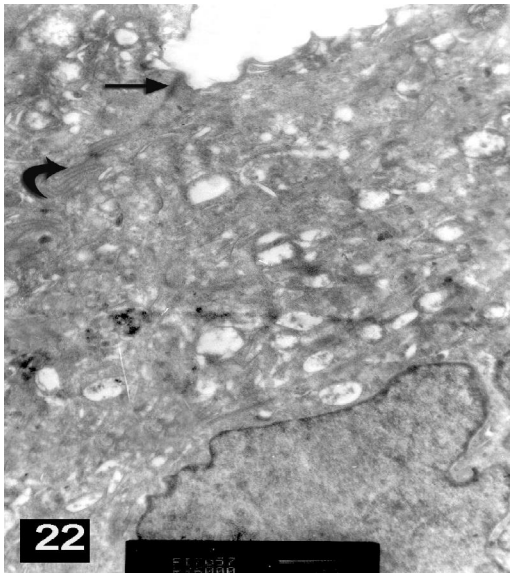

Figure (22): An electron micrograph from the urinary bladder of the Tibolone treated ovariectomized rats showing the tight junction (arrow) with prominent interdigitations (curved arrow) in the adjacent lateral surfaces of superficial cells. (X13000).

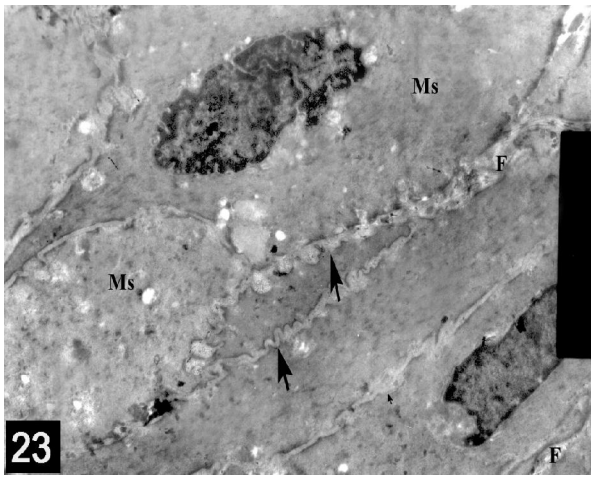

Figure (23): An electron micrograph from the urinary bladder of the Tibolone treated ovariectomized rats showing straightsarcolemmae with distinct dense bodies and plaquessmooth muscle cells (Ms)**.** Sarcolemmae of some myocytes are corrugated (arrows) with minimal intercellular spaces containing some collagen fibers (F). (X11000).

4.Discussion

Since the urinary and reproductive systems have a common embryologic origin in the urogenital sinus they are linked not only anatomically, but also functionally. The fact that estrogen receptors are located mainly throughout the female genital tract, bladder, urethra and pelvic floor, supports their essential role in the maintenance of physiologic activity in female urogenital tract **[23]**.

In this study, the bladder of the ovariectomized rats revealed an observable focal reduction in the epithelial lining and the total thickness of the bladder wall in comparison with that observed in the control group. Ultrastructurally, marked reduction in the urothelium layers were observed. These results were confirmed by statistical analysis that clarified decrease in the urothelium and bladder wall thickness as compared with the control group. It had been stated **[24,25]** that the bladder mucosa has a significantly higher rate of glycolysis, oxidative metabolism and greater mitochondrial enzyme activity. So, the bladder urothelium is extremely sensitive to decrease in the blood flow after ovariectomy. This urinary bladder ischemia led to significant hypoxia and atrophy of the mucosal lining which may be accompanied by increased mucosal permeability. So, low circulating estrogen has been linked to urinary bladder dysfunction. In contrary**[26]**,no major changes were found in bladder wall thickness and this attributed to the short duration of the study. The severity of the bladder changes being directly correlated with the duration of hormonal deprivation.

In ovariectomized rats, some epithelial cells had vacuolated cytoplasm with peripherally placed nuclei. Few cells had deeply stained nuclei. Some researchers documented**[14,27,28]**thatan increase of apoptotic activity in the urothelium leading to reduction in urothelial thickness. Healthy young urothelium possesses a powerful antioxidant defense system that is composed of numerous antioxidants. Moreover **[26,29,30]**, estrogen and estrogen metabolites are known to act as endogenous antioxidants protecting the membrane phospholipids from lipid peroxidation. They have an anti-apoptotic effect through expression of bcl-2 and an inhibitory effect on bax protein expression which was significantly elevated in the affected urothelium. So, estrogen deficiency is correlated with a concurrent increase in hypoxic or oxidative damage in the urothelium. In addition**[31,32]**, urothelial cells of aging and ischemic bladder showed marked decrease in total antioxidant capacity and significant increased levels of lipid peroxides as markers of oxidative stress.

In the current work, desquamated epithelial cells were observed within the lumen. Areas of complete denudation of the mucosa with exposed lamina propria were also encountered. Ultrastructurally, indistinct epithelial cell junctions were also observed. It was reported **[33,34]** that alteration in cell junctions leading to desquamation of epithelial cells. So, the urothelium loses its function as a barrier in distinct areas as a consequence of cellular detachment due to this desquamation or apoptosis. The remaining basal cells in the intact areas proliferate and differentiate as a trial to increase the thickness of the urothelium.

In this work, ovariectomized bladder showed wide intercellular spaces between urothelial cells. Infiltrating inflammatory cells contained numerous variable sized electron dense granules were detected in urothelium. It was reported **[35]**that the bladder mucosa is impermeable in nature and acts as a barrier in protecting the bladder from damage by toxic substances. The high incidence of bladder infection during menopause due to marked reduction of glycosaminoglycansin the epithelium which considered as a component of urothelium barrier. Some scientists **[23]** stated that urothelial atrophy secondary to bilateral ovariectomy is a fact suggested by wide intercellular spaces and loss of intercellular junctions. Urothelial atrophy is followed by decreased barrier function that may be predisposed to inflammation. Foci of many inflammatory cells were present in the lamina propria and sometimes immediately underlying the urothelium. Additionally, significant increase in mast

cells number was explained by the presence of estrogen receptors that directly influence their secretion and degranulation.

Musculosa of the ovariectomized rats showed distorted arrangement. Most of smooth muscle cells were darkly stained and showed indistinct dense bodies and plaques**.** Their sarcolemmae were markedly corrugated. Some researchers**[31]** stated that ovariectomy reduce blood flow to the bladder smooth muscle resulting in hypoxia and subsequent structural and contractile changes. This reduction was attributed by other authors**[1]**to the marked decrease of vascular endothelial growth factor that responsible for decrease angiogenesis and number of blood vessels leading to muscular weakness. The opinion of other researchers **[25,36]** that the reduction in the contractile function of the smooth muscle was mediated by decreased mitochondrial enzymatic activity, decreased calcium storage and changes in regulatory proteins. Furthermore**[20],**the corrugated plasma membrane, decrement in the myofilaments, dense bodies and plaques may be involved in marked reduction in the bladder contractility.

Also, the musculosa of ovariectomized rats showed widened intercellular spaces containing abundant collagen fibers. Morphometrical and statistical analysis revealed decrease in area % of smooth muscle (SM) with increase in area % of collagen fiber (CF) and CF/SM ratio as compared with the control group. Some authors **[37]**mentioned that although collagen is ordinary produced by fibroblasts, nonumerous fibroblasts were detected. There were fibroblast-like muscle cells can actively produce collagen fibers. However, others **[24]**attributed collagen fibers hyperplasia tofibroblasts hyperplasia that subsequently increases collagen synthesis. Itwas described **[20]**that the ratio of smooth muscle to connective tissue is vital for proper bladder contraction and relaxation. The aggregated collagen fibers in the widened spaces between muscle fascicles; collagenosis lead to stiffening of bladder wall whichmay be the main cause of voiding dysfunction in estrogen deficit.

Estrogen replacement therapy (ERT) improves the climacteric complaints and in turn, quality of life. But ERT was terminated by the Women's Health Initiative (WHI) because of increased risk of stroke and breast cancer. Therefore, alternative therapy like Tibolone is attractive option thatneed to be evaluated **[38]**.

In our study examination of the empty urinary bladder of the Tibolone treated ovariectomized rats revealed results similar to that observed in estrogen replacement therapy studies.

In this work, urothelium as well as total bladder wall thickness of Tibolone treated ovariectomized group was relatively increased when compared with ovariectomized one. Although the urothelium became multilayered, its cells were disorganized. The adjacent lateral surfaces of superficial cells were joined by tight junction with prominent interdigitations. These results were confirmed by statistical analysis that clarified increase in the urothelium and bladder wall thickness as compared with the ovariectomized one approaching control level. It was explained**[39]** that estrogen has a protective effect on the integrity of the urothelium due to increase the blood flow, vascular density and angiogenesis in the bladder's wall. It increases the urothelial thickness not only as a result of increase cell activity **[40]**but also by suppression of baxgene expression**[41].**Others **[29]** reported that estrogen stimulates prostaglandins synthesis that preserves bladder mucosal integrity by promoting epithelial cell growth, closure of epithelial tight junctions and maintain the protective glucosaminoglycans in the superficial layer of the urothelium.

Also, in the Tibolone treated group, all urothelial cells were nearly close to each other and contained many vacuoles. Similar findings **[14]** were detected with estradiol therapy which leading to the appearance of large vacuoles within the urothelium. Although the effect of epithelial vacuolar change on bladder function is still unresolved, it may be related to the affection of bladder epithelial permeability or barrier function.

The smooth muscle bundles appeared in different directions. Most of muscle cells had pale stained cytoplasm. Few ones were still deeply stained. Ultrastructurally, they had straight sarcolemmae with distinct dense bodies and plaques**.** Sarcolemmae of some myocytes were corrugated with minimal intercellular spaces containing few collagen fibers. Morphometrical and statistical analysis revealed increase in area % of smooth muscle (SM) with decrease in area % of collagen fiber (CF) and CF/SM ratio as compared with the ovariectomized one approaching control level. Increased smooth muscle layer thickness and density of the bladder was attributed to either increased expression of estrogen receptor- β **[42]**or specific growth factors, such as basic fibroblast growth factor and transforming growth factor-β **[14].** Estrogen can also enhance the detrusor cell regulatory proteins, which in turn upregulates the actin–myosin interaction and contraction in detrusor muscles**[43]**. It was stated alsothat estrogen supplementation could improve bladder function through inhibition of collagen hyperplasia by preventing the proliferation of fibroblasts**[44,45]**or elevated the expression of vascular endothelial growth factor in urothelium and endothelial cells. This factorenhanced smooth muscle formation and angiogenesis, although, it had been found mild atrophy

and fibrosis of the detrusor muscles persisted after estradiol**[19]**.

Tibolone is a synthetic steroid with tissue selectivity that has progestogenic, some androgenic as well as estrogenic effects. It is a selective tissue estrogenic activity regulator because it acts as an estrogen on brain, vagina and bone but not on endometrium and breast. So, it protects bone, alleviates hot flashes, improves both vaginal atrophy and urogenital symptoms without having an estrogen-likestimulating effect on the endometrium or breast. Consequently, it is less risky than estrogen-based therapies**[46,47]**.

Conclusion:

From these results we concluded that estrogen deficiency led to deterioration of bladder morphology. Tibolone is considered a good therapy for estrogen deficiency in improving bladder morphology.

References

1. **Yu Y, Shen Z, Zhuo X and Chen S.** Effects of steroid hormones on morphology and vascular endothelial growth factor expression in female bladder. Urology 2009; 73: 1210 –1217.
2. **Saxena P, Prakash A, Nigam A and Mishra A.** Polysystic ovary syndrome: Is obesity a sine qua non? A clinical, hormonal, and metabolic assessment in relation to body mass index. Indian J Endocrinol Metab 2012; 16(6):996-999.
3. **Osborne CK, Zhao H and Fuqua SA.** Selective estrogen receptor modulators: Structure, function and clinical use. J Clin Oncol 2000; 18:3172-3186.
4. **Faria TDS, Soares LL, Medeiros JL, Boaventura GT, Sampaio FJB and Ramos CF.** Morphological modification of female bladder after prolonged use of soy-based diets.Maturitas 2009; 62: 42-46.
5. **Harlow BL & Signorello LB.** Factors associated with early menopause. Maturitas 2000; 35: 3-9.
6. **Henn EW.** Menopause and its effect on the female lower urinary tract. SA Fam Pract 2010; 52(5):405-408.
7. **Chang SH, Kim CS, Lee KS, Kim H, Yim SV, Lim YJ and Park SK.** Premenopausal factors influencing premature ovarian failure and early menopause. Maturitas 2007; 58:19-30.
8. **Shuster LT, Rhodes DJ, Gostout BS, Grossardt BR and Rocca WA.** Premature menopause or early menopause: Long term health consequences. Maturitas 2010; 65 (2): 161-172.
9. **Dell'Edera D, Tinelli A, Capozzi O, Epifania AA, Malvasi A, Lofrese D, Pacella E, Milazzo GN, Mazzone E, Leo M and Rocchi M.** Clinical correlation between premature ovarian failure and a chromosomal anomaly in a 22-year-old Caucasian woman: A case report. Journal of Medical Case Reports 2012; 6:368-373.
10. **Kalantaridou SN, Naka KK, Papanikolaou E, Kazakos N, Kravariti M, Calis KA, Paraskevaidis EA, Sideris DA, Tsatsoulis A, Chrousos GP and Michalis LK.** Impaired endothelial function in young women with premature ovarian failure: Normalization with hormone therapy. The Journal of Clinical Endocrinology & Metabolism 2004; 89(8):3907–3913.
11. **Atsma F, Bartelink ML, Grobbee DE and van der Schouw YT.** Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: A meta-analysis. Menopause 2006; 13(2): 265-279.
12. **Gallagher JC**. Effect of early menopause on bone mineral density and fractures. Menopause 2007; 14: 567–571.
13. **Palacios S.** Advances in hormone replacement therapy: Making the menopause manageable. BMC Womens Health 2008; 8: 22-27.
14. **Aikawa K, Sugino T, Matsumoto S, Chichester P, Whitbeck C and Levin RM.** The effect of ovariectomy and estradiol on rabbit bladder smooth muscle contraction and morphology. The Journal of Urology 2003; 170: 634–637.
15. **Bortolini MAT, Silva ID, Hamerski MG, Castro RA, Sartori MG and Girao MJ.** Influence of ovarian hormones deprivation on gene expression in the lower urinary tract of rats. International Braz J Urol 2007**;** 33(4): 544-553.
16. **Modelska K and Cummings S.** Tibolone for postmenopausal women: Systemic review of randomized trials. The Journal of Clinical Endocrinology & Metabolism 2002; 87(1):16–23.
17. **Blom MJ, Wassink MG, De Gooyer ME, Ederveen AGH, Kloosterboer HJ, Lange J, Lambert JGD and Goos HJTh.** Metabolism of Tibolone and its metabolites in uterine and vaginal tissue of rat and human origin. J Steroid Biochem Mol Biol 2006; 101: 42-49.
18. **Verheul HAM, van Iersel MLPS, Delbressine LPC, Kloosterboer HJ**. Selective tissue distribution of Tibolone metabolites in mature ovariectomized female cynomolgus monkeys after multiple doses of Tibolone. The American Society for Pharmacology and Experimental Therapeutics 2007; 35:1105–1111.
19. **Çayan F, Tek M, Balli E, Öztuna S, Karazindiyanoğlu S and Çayan S.** The effect of testosterone alone and testosterone + estradiol therapy on bladder functions and smooth muscle/collagen content in surgically menopause induced rats. Maturitas 2008; 60: 248–252.
20. **Xin Y, Ya-zhen L, Zhuo M, Pei G and Ming S.** Effect of estrogen and Tibolone on bladder histology and estrogen receptors in rats. Chinese Medical Journal 2009; 122(4):381-385.
21. **Bancroft J. and Gamble A.** Theory and Practice of Histology Techniques. 6th ed., Pp: 83-92, Churchil Livingstone, New York and London, 2008.
22. **Singh D.** Principle and Techniques. In Histology, Microscopy and Photography. 1st ed., Pp: 679-699, CBS Publishers and Distributers. New Delhy, Bangalore (India), 2003.
23. **Lucan L, Lucan VC, Tabaran FA and Stamatian F.** Changes in the urinary bladder morphology, mast cell population and estrogen receptor alpha expression following ovariectomy and chronic estrogen

replacement therapy in albino rats. Acta Endocrinologica 2013; XI(1):11-22.

24. **Lin WY, Radu F, Schuler C, Leggett RE, Mannikarottu A and Levin RM.** The effect of ovariectomy and estrogen therapy on free fatty acid content, endogenous lipase activity and phospholipid content of the rabbit urinary bladder.BJU Int 2008; 102: 885-889.
25. **Li S, Hydery T, Juan Y, Lin WY, Kogan B, Mannikartottu A, Leggett RE, Schuler C and Levin RM.** The effect of 2- and 4- week ovariectomy on female rabbit urinary bladder function. Urology 2009; 74: 691–697.
26. **Lucan L, Stamatain F, Lucan V and Tabaran AF.** Cell proliferation and apoptosis in urinary bladder urothelium of rats following ovariectomy and chronic estrogen replacement therapy. Clujul Medical 2013; 86(1): 27-30.
27. **Ohata R.** Effect of estrogen replacement therapy on the expression of apoptosis-related genes in old female rats. Yonago Acta Medica2002**;** 45: 95-102.
28. **Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M and Telser J.** Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39(1): 44-84.
29. **Hass MA, Nichol P, Lee L and Levin RM.** Estrogen modulates permeability and prostaglandin levels in the rabbit urinary bladder. Prostaglandins, Leukotrienes and Essential Fatty Acids2009**;** 80: 125–129.
30. **Berens HM and Tyler KL.** Proapoptotic Bcl-2 protein plays an important role in the pathogenesis of Reovirus Encephalitis. J Virol2011; 85(8): 3858-3871.
31. **Juan YS, Mannikarottu A, Kogan BA, Leggett RE, Whitbeck C, Chichester P, Lin WY, Johnson A and Levin RM.** The effect of low dose estrogen therapy on ovariectomized female rabbit bladder.Urology 2008; 71: 1209 –1213.
32. **Perše M, Injac R and Erman A.** Oxidative stress and lipofuscin accumulation in urothelial cells of bladder in aging mice. PLoS One 2013; 8(3): 1-11.
33. **Jezernick K, Medalia O and Arnson M.** A comparative study of the desquamation of urothelial cells during gestation and in adult mice following moderate stress or endotoxin treatment. Cell Biol Int 1995; 19:887-893.
34. **Jezernik K, Romih R and Veranic P.** Urothelial cell detachment and differentiation in urinary bladder. Pflugers Arch Eur J Physiol 2000; 439: 135-136.
35. **Ponglowhapan S, Church DB and Khalid M.** Effect of gonadal status and gender on glycosaminoglycans profile in lower urinary tract of dogs. Theriogenology 2011; 76: 1284–1292.
36. **Kubota Y, Hashitani H, Fukuta H, Kubota H, Kohri K and Suzuki H.** Role of mitochondria in generation of spontaneous activity in detrusor smooth muscles of guinea pig bladder. J Urol2003**;** 170(2): 628-633.
37. **Al-Motabagani MA.** Age related changes in the urinary bladder of the female albino rats. Int J Morphol 2005; 23(4): 309-316.
38. **Somunkiran A, Erel CT, Demirci F and Senturk ML.** The effect of Tibolone versus 17-estradiol on climacteric symptoms in women with surgical menopause: A randomized, cross-over study**.** Maturitas 2006; 56: 61–68.
39. **Battaglia C, Salvatori M, Giulini S, Primavera MR, Gallinelli A and Volpe A.** Hormonal replacement therapy and urinary problems as evaluated by ultrasound and color Doppler. Ultrasound Obstet Gynecol 1999; 13(6):420-424.
40. **Hextall A and Cardozo L.** The Effect of Estrogen and Antiestrogens on the Urogenital Tract. Handbook of Experimental Pharmacology Part 4, Pp: 363-377, Springer Berlin Heidelberg, 1999.
41. **Suguita M, Girão MJBC, Simões MJ, Sartori MGF, Baracat EC and Rodrigues G.** A morphologic and morphometric study of the vesical mucosa and urethra of castrated female rats following estrogen and/or progestogen replacement. Clin Exp Obstet Gynecol 2000; 27:176–178.
42. **Makela S, Strauss L, Kuiper G, Valve E, Salmi S, Santti R and Gustafsson JA.** Differential expression of estrogen receptors alpha and beta in adult rat accessory sex glands and lower urinary tract. Mol Cell Endocrinol 2000; 170;219-229.
43. **Lin AD, Levin RM, Kogan BA, Whitbeck C, Keam C and Mannikarottu A.** Alteration of contractile and regulatory proteins in estrogen-induced hypertrophy of female rabbit bladder. Urology 2006; 68:1139-1143.
44. **Fleischmann N, Christ G, Sclafani T and Melman A.** The effect of ovariectomy and long-term estrogen replacement on bladder structure and function in the rats.The Journal of Urology 2002**;** 168: 1265–1268.
45. **Lin AD, Levin R, Kogan B, Whitbeck C, Chichester P, Sokol R and Mannikarottu A.** Estrogen induced functional hypertrophy and increased force generation of the female rabbit bladder. Neurourol Urodyn 2006; 25(5): 473-479.
46. **Sharma S, Mahajan A, Kumar S, TandonVR.** Tibolone: A selective tissue estrogenic activity regulator. Drug Review. JKScience 2006**;** 8(4): 229- 230.
47. **Jacobsen DE, Samson MM, Schouw YT, Grobbee DE and Verhaar HJ.** Efficacy of Tibolone and raloxifene for the maintenance of skeletal muscle strength, bone mineral density, balance, body composition, cognitive function, mood/depression, anxiety and quality of life/well-being in late postmenopausal women ≥ 70 years: Study design of a randomized, double-blind, double-dummy, placebo-controlled, single-center trial. Trials 2008; 9: 32-41.